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PRESERVATION OF THE CHONDROCYTES PERICELLULAR MATRIX IMPROVES CELL-INDUCED CARTILAGE FORMATION

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Purpose: Chondrocytes are often used for cartilage tissue engineering. However, in native cartilage, the chondrocytes are surrounded by a pericellular matrix, together forming the chondron. Since cells are influenced by their surroundings, we hypothesized that retaining the pericellular microenvironment would influence the synthetic capacity of the chondrocytes. Therefore the aim of this study was to investigate whether the pericellular matrix has an effect on cell-induced cartilage formation.
Methods: Chondrocytes and chondrons isolated from nucleus pulposus (NP), annulus fibrosus (AF), and articular cartilage (AC) from goats, were cultured for 25 days in alginate beads. After 7, 18 and 25 days of culture, the amount of proteoglycans present in the alginate beads was measured and collagen was extracted from the beads. Immunoblotting for type II collagen was performed on the collagen extracted from the alginate beads. Protein expression of matrix metalloproteinase 2 (Mmp2) and Mmp9 was analyzed by zymography and gene expression levels of Mmp13 were measured by real-time PCR.
Results: Chondrons and chondrocytes were successfully isolated from AC, AF, and NP. The amount of proteoglycans found in the alginate beads was significantly higher in the chondrons from AC and NP compared to the chondrocytes, but no differences were found between chondrons and chondrocytes from AF. The type II collagen that was extracted from the alginate beads containing the chondrons from all the cartilage sources was cross-linked, whereas the type II collagen produced by the chondrocytes consisted only of non-crosslinked alpha1 (II) chains. Both Mmp2 and Mmp9 expression were higher by
the chondrocytes from AC and NP compared to the chondrons, no differences were found with the AF cells. At day 0 the gene expression levels of MMP13 were low in both chondrocytes and chondrons. However, after 18 and 25 days of culture, there was a significant increased expression by the chondrocytes and not by the chondrons.
Conclusions: This study shows that maintaining the native chondrocytes pericellular matrix affects both anabolic and catabolic activities. The cross-links present in the type II collagen produced by the chondrons isolated from all the different tissues suggests that the pericellular matrix has an effect on the expression or the activity of enzymes involved in collagen cross-linking. The type II collagen produced by the chondrons does more resemble the collagen found in the native tissues. It is also likely that the altered cell-ECM interactions caused by removal of the pericellular matrix plays a role in the increased expression of the matrix metalloproteinases.
Taken together, our data suggest that the extracellular matrix surrounding the chondrocytes is essential for maintaining its proper composition and that preserving the thin matrix layer surrounding the chondrocytes improves cell-induced hyaline cartilage formation.

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HISTONE DEACETYLASE INHIBITORS AS CHONDROPROTECTIVE AGENTS

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Purpose: Cartilage destruction in osteoarthritis is thought to be mediated mainly by the action of proteinases from the matrix metalloproteinase (MMP) and 'a disintegrin and metalloproteinase domain with thrombospondin motifs' (ADAMTS) families. The expression of these enzymes can be altered through changes in protein acetylation mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Cell-based and cartilage explant assays show that HDAC inhibitors can block proinflammatory cytokine induction of key MMPs and ADAMTSs resulting in inhibition of cartilage resorption. This project aims to define the molecular pathways by which HDAC inhibitors mediate their chondroprotective affects.
Methods: The activity of trichostatin A (TSA), valproic acid (VPA) and MS-275 on histone and α-tubulin acetylation within SW1353 cells was assessed via western blot. Quantitative RT-PCR was used to determine the effect of HDAC inhibitors on cytokine induced metalloproteinase expression. Bovine nasal cartilage (BNC) explant assays were used to determine the effect of HDAC inhibitors on cartilage destruction.
Results: Western blot analysis concluded all inhibitors cause a concentration dependent increase in histone 3 and histone 4 acetylation, with only trichostatin A causing an increase in α-tubulin acetylation. Quantitative RT-PCR analysis of cytokine stimulated SW1353 cells showed that MMP1 and MMP13 expression is significantly reduced by TSA and VPA but not MS-275, and all three compounds significantly reduce cytokine induced MMP3 expression. BNC explant assays indicate all three compounds inhibit cytokine induced cartilage resorption and we are currently screening gene expression in this model.
Conclusions: All three inhibitors protect against cytokine induced cartilage resorption despite their differential ability to inhibit classical HDACs. MS-275 blocks cartilage resorption despite having no effect on the expression of key collagenase genes. In common with the other HDAC inhibitors, it does abrogate cytokine-induced MMP3 expression, potentially preventing collagenase activation, and this may therefore be a key mode of action for such compounds.

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ACUTE-PHASE AGGRAVATION OF CHONDROCYTE DEATH IN HUMAN ANKLE INTRAARTICULAR FRACTURES

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Purpose: In intraarticular fractures (IAFs), death and dysfunction of chondrocytes on the fractured articular surface presumably play an important role in subsequent cartilage degeneration that eventually leads to post-traumatic OA. In this study, cell-level pathology of fracture-associated cartilage injury and its time-dependent changes were explored in a quasi-*in-vivo* model of human ankle IAF. It was hypothesized that chondrocyte death associated with IAF would be concentrated near fracture edges, and that that cartilage damage would aggravate with time in the acute phase.
Methods: Four normal human ankles immediately ($<$ 4hrs) following surgical amputation, from femoral malignant tumor patients (29-57y, 2M, 2F), were subjected to fracture insult in which the most typical injury mechanism of clinical distal tibial "pilon"